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PRINCIPAL INVESTIGATOR: Jeffrey K. Griffith, Ph.D.

CONTRACTING ORGANIZATION: University of New Mexico Health Sciences Center
Albuquerque, New Mexico 87131

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13. ABSTRACT (Maximum 200 Words) The goal of this project is to test the hypothesis that the content of telomere DNA in clinically localized prostate cancers has independent prognostic significance. If so, then telomere DNA content may discriminate high-risk patients that are most likely to benefit from therapy from low risk patients who can be spared unnecessary side effects and expense by management with "watchful waiting". This hypothesis is being tested by comparing telomere DNA content in archival prostate cancer tissue from surviving and non-surviving men who received prostatectomies for clinically localized prostate cancer between 1986 and 1992. During the past year, 110 men have been entered into the study group, including 24 men who died from prostate cancer, 4 surviving men that had received orchiectomy for serious recurrent disease (poor outcomes) and 82 men who have remained disease-free (good outcomes). Archival tissue blocks have been obtained for 65 members of the study group and examined histologically. DNA has been extracted from new sections and initial telomere DNA content assays have been performed on tissue from 40 of these men. Although still preliminary, the data is consistent with the hypothesis that telomere DNA content is correlated to outcome in prostate cancer ($p < 0.0001$).				
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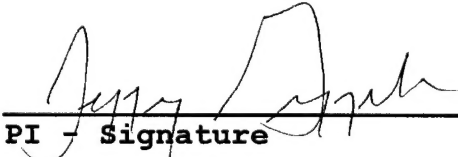
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INTRODUCTION:

The lifetime risk of being diagnosed with prostate cancer is about 40%. However, only 10% of these men will develop clinical cancers and only 3% will die from prostate cancer. Since there are no factors that readily identify a newly diagnosed prostate cancer as being aggressive, all men with clinically localized disease will be considered for curative therapy, even though this treatment will only benefit a minority of patients. These treatments carry a small risk of mortality, but substantial risks of impotence, incontinence and bowel problems. Furthermore, over 40% of clinically localized cancers are upstaged at the time of surgery, and are not curable. Therefore, the goal of this project is to develop markers of prostate tumor aggressiveness that can discriminate the high-risk patients who are most likely to benefit from these therapies from low risk patients who can be spared their unnecessary side effects and expense by management with "watchful waiting". The experiments specifically test the hypothesis that the content of telomere DNA in clinically localized prostate cancers has independent prognostic significance. This hypothesis is being tested by comparing telomere DNA content in archival prostate cancer tissue from surviving and non-surviving men who received prostatectomies for clinically localized prostate cancer between 1986 and 1992.

BODY OF ANNUAL PROGRESS REPORT:

This section describes progress associated with each task outlined in the approved Statement of Work.

Task 1: *Months 1-2. Design and perform database search of NMTR to identify 400 surviving and non-surviving men who received prostatectomies for advanced prostate cancer between 1986 and 1988.*

Status: The initial experimental design involved a study group comprised of 150 surviving and 150 non-surviving men. This design, which was based on estimated telomere DNA contents in the two groups, would provide a significance level of 0.05 and a power 0.8. However, our subsequent experiments demonstrate that there is a two-fold, and highly significant ($p < 0.0001$), difference between the telomere DNA contents in prostate cancers from surviving and non-surviving men (Table 2, Figures 1,2: Donaldson et al., 1999). Based on these results, we calculate that approximately 50 surviving and non-surviving men will provide a significance level of 0.05 and a power 0.8. These results strongly support the initial hypothesis.

The New Mexico Tumor Registry has been searched to identify men with and without recurrent prostate cancer who received prostatectomies between 1986 and 1992. To date, a total of 110 men from three local hospitals have been entered into the study group. The study group currently includes 24 men who died from prostate cancer, 4 surviving men that had received orchiectomy for serious recurrent disease (poor outcomes) and 82 men who have remained disease-free (good outcomes). Many additional "good outcome" men also were identified who will be entered into study, as necessary. Approximately 20 more men who died from prostate cancer have been identified at three other hospitals and will be entered into the study, as necessary, contingent upon approval of these hospitals' IRBs. We are also attempting to identify additional "poor outcome" patients by reviewing surviving patients' records for progressively increasing levels of

prostate-specific antigen (PSA). Therefore, we have identified a sufficient number of "good outcome" men, and almost enough "poor outcome" men for the analysis.

Task 2: *Months 3-24. Archival tissue blocks derived from the 400 selected cases will be identified, requested and collected from New Mexico hospital pathology departments by NMTR staff.*

Status: Archival tissue blocks (both tumor and peripheral normal tissue) have been obtained for 65 members of the new 100 member study group and confirmed histologically.

Task 3: *Months 3-6. Ms. Magotra-Clerc will learn histological and micro-dissection techniques from Dr. Joste.*

Status: Ms. Magotra-Clerc left the graduate program for personal reasons and is no longer a member of this laboratory. Her duties have been reassigned to Mr. Curtis Hines and Ms. Colleen Fordyce, second and third year Ph.D. students, respectively.

Task 4: *Months 6-24. New sections will be cut from approximately 304 blocks of paraffin-embedded tissue, examined microscopically and micro-dissected to separate tumor cells from contaminating stromal cells and connective tissue. DNA will be extracted concurrently and stored for analysis.*

Status: Instead of micro-dissecting tissue blocks, we have found that it is sufficient to separate histologically confirmed prostatic adenocarcinoma from peripheral normal tissue by gross dissection. Briefly, slides corresponding to archival blocks are examined microscopically to identify blocks that contain the highest percentage of tumor cells (typically >75%). Tissue sections 25 microns thick are then cut from the blocks, and histologically confirmed adenocarcinoma is separated from peripheral prostate tissue by gross dissection of tissue blocks guided by slides, as necessary (Donaldson et al., 1999). The same approach is also used to obtain peripheral normal tissue.

Sections containing tumor and peripheral normal tissue have been cut from 65 specimens of the new 100 member study group (130 total samples) and DNA has been extracted.

Task 5: *Months 9-26. Initial telomere DNA content assays will be performed on the 304 DNA samples from the micro-dissected normal and tumor tissues.*

Status: Initial telomere DNA content assays (Bryant et al, 1997) have been performed on 80 samples, comprised of paired tumor and peripheral normal tissue from 40 men in the new 100-member study group. These initial analyses, and our parallel analysis of breast cancer tissues (Griffith et al, 1998) , revealed an unexpected and highly important problem. This is the need to remove all extraneous paraffin from the section, especially when tissue is scant, as it results in artificially elevated telomere DNA content. Accordingly, extraneous paraffin is now removed with scalpels after the tissue is sectioned, thus preserving the original tissue block for future studies.

Each sample of tumor and peripheral normal tissue DNA is serially diluted three times and each dilution is assayed in parallel by our slot blot technique (Bryant et al, 1997). The coefficient of variation for the data obtained from the three dilutions is calculated for each DNA sample, and must be less than 20%. DNA extracted from each sample is assayed independently a minimum of three times. The mean telomere DNA content is determined from the replicate experiments. Telomere DNA contents of 'good outcome' and 'poor outcome' patients are compared using a paired t-test or Wilcoxon non-parametric test.

Task 6: Months 27-30. Ambiguous assays will be repeated as necessary (estimated at 20% of initial analyses).

Status: No Activity

Task 7: Months 27-30. Perform statistical analysis of the collected data.

Status: No Activity

Task 8: Months 27-30. Prepare and submit manuscript and final report.

Status: No Activity

KEY RESEARCH ACCOMPLISHMENTS:

- Published study correlates telomere DNA content with prognosis in localized prostatic carcinoma.
- Determination that excess paraffin artifactually elevates apparent telomere DNA contents.
- Development of a database that includes all study subjects' vital statistics and current status.
- Development of an objective means for data analysis.

REPORTABLE OUTCOMES:

Articles Published :

L. Donaldson, C.F. Fordyce, A. Smith, F. Gilliland, R.K. Moyzis and J.K. Griffith. *Association between outcome and telomere DNA content in prostate cancer*. Journal of Urology 162: in press.

Funding Applications:

NIH: *Molecular Markers of Prostate Cancer Progression*. Objective: To assess the relationship between c-myc amplification, telomere DNA content and telomerase expression in prostate cancer.

NIH: *Prognostic Value of Telomere DNA in Prostate Biopsy*. Objective: To determine the feasibility of extending application of our telomere DNA content slot blot assay to prostate needle-core biopsy specimens.

Invited Seminars, Meetings and Presentations:

1st Annual Women's Health Research Symposium, Albuquerque, NM, 1999
Association of American Medical and Graduate Departments of Biochemistry, Puerto Vallarta, Mexico, 1999

Departmental Seminars and Continuing Medical Education:

UNM SOM Department of Biochemistry and Molecular Biology, 1999
UNM SOM Department of Molecular Genetics and Microbiology, 1999
UNM SOM Department of Pathology, 1998

Awards:

Ms. Colleen Fordyce, a Ph.D. student working on this project, received the *1999 Pfizer Scholars in Urology Award* for outstanding achievement in the advancement of urological science at the UNM School of Medicine.

CONCLUSIONS:

This study investigates the relationship between telomere DNA content and outcome in prostatic adenocarcinoma. Overall, we have made good progress toward completing the tasks in the approved Statement of Work. Although still preliminary, the data is consistent with our earlier investigations which demonstrated that telomere DNA content is correlated to outcome in prostate cancer ($p < 0.0001$). Given that the lifetime risk of being diagnosed with prostate cancer is about 40%, and that prostate cancer has two apparent phenotypes, one which is highly aggressive and one that is indolent, it is important to develop markers of tumor aggressiveness. Our current assay may be adapted for use on biopsy specimens; making it possible for a physician to accurately assess the severity of a patient's prostate cancer at diagnosis and implement a treatment particularly suited to each patient's disease.

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- J.E. Bryant, K.G. Hutchings, R.K. Moyzis and J.K. Griffith (1997) *A sensitive measure of telomere DNA content in human tissue*, **Biotechniques**, 23:476-484.
- L. Donaldson, C.F. Fordyce, A. Smith, F. Gilliland, R.K. Moyzis and J.K. Griffith (1999) *Association between outcome and telomere DNA content in prostate cancer*. **Journal of Urology**. 162: *in press*.
- J.K. Griffith, J.E. Bryant, C. Fordyce, F. Gilliland N. Joste, and R.K. Moyzis (1999) *Reduced telomere DNA content is correlated with genomic instability and metastasis in invasive human breast carcinoma*. **Breast Cancer Research and Treatment**, 54: 59-64.

LIST OF APPENDICES:

Preprint:

L. Donaldson, C.F. Fordyce, A. Smith, F. Gilliland, R.K. Moyzis and J.K. Griffith
(1999) *Association between outcome and telomere DNA content in prostate cancer.*
Journal of Urology. 162: *in press.*

ASSOCIATION BETWEEN OUTCOME AND TELOMERE DNA CONTENT IN PROSTATE CANCER

LESLIE DONALDSON, COLLEEN FORDYCE, FRANK GILLILAND, ANTHONY SMITH,
RICHARD FEDDERSEN, NANCY JOSTE, ROBERT MOYZIS AND JEFFREY GRIFFITH*

From the Department of Biochemistry and Molecular Biology, the Department of Medicine, the Department of Surgery, and the Department of Pathology, University of New Mexico School of Medicine, Albuquerque, and the Center for Human Genome Studies, Los Alamos National Laboratory, Los Alamos, New Mexico

ABSTRACT

Purpose: To perform an initial retrospective investigation of the relationship between outcome in patients with organ confined prostate adenocarcinoma and the tumor cells' content of telomere DNA.

Materials and Methods: The case-controlled study group was composed of eighteen men diagnosed with prostatic adenocarcinoma prior to 1993. The group was selected so that approximately one half died within ten years of diagnosis and one half survived ten years or longer. Archival, paraffin-embedded tumor tissue was recovered for each patient. DNA was extracted from newly cut sections, fixed to nylon membranes and hybridized with P³²-labeled centromere- and telomere-specific probes. Telomere DNA contents were quantitated from the hybridized radioactivities. The relationships between telomere DNA content and survival, and telomere DNA content and disease recurrence in men receiving prostatectomies were determined.

Results: Death and disease recurrence were associated with reduced telomere DNA content ($p < 0.0001$, $p < 0.0001$, respectively).

Conclusions: Telomere DNA content may differentiate high-risk patients with metastatic prostate cancer from men with indolent disease who can be spared the unnecessary side effects and expense of treatment by management with "watchful waiting."

KEY WORDS: metastasis, outcome, prognosis, prostate cancer, recurrence, telomere

Prostate cancer causes approximately 40,000 deaths annually in the United States.¹ However, only about one in 16 men with clinically diagnosed prostate cancer dies of his disease. Autopsy studies indicate that 40% of men over the age of 50 have undiagnosed invasive prostate cancer, implying that over nine million men have latent disease.² The treatment options for men who require medical intervention—radical prostatectomy, radiation therapy, and androgen deprivation—have severe complications, including incontinence, urethral stricture, impotence and mortality. Therefore it is important to develop markers which discriminate the less common, high-risk patients who are most likely to benefit from aggressive therapy from the more common patients with indolent disease who can be spared the unnecessary side effects and expense of treatment by management with "watchful waiting."

The genotypic differences between lethal metastatic prostate cancers and less-threatening indolent cancers are not known. However aneuploidy, the gain or loss of genomic DNA, is ubiquitous in lethal prostate cancer. Comparative genomic hybridization has revealed that DNA is duplicated on the 8q chromosome arm and deleted from the 8p, 13q, 16q, 17q and 10q arms in 50%–85% of metastatic prostate cancer samples examined,³ implying a causal relationship between genomic instability and the evolution of the aggressively growing, metastatic phenotype.

Telomere shortening is one mechanism that has been pro-

posed to produce phenotypic variability in cancer cells.⁴ Human telomeres are comprised of 1,000–2,000 tandemly repeated copies of the hexanucleotide TTAGGG.⁵ Due to incomplete replication of terminal DNA sequences, and the absence of telomerase, the ribonucleoprotein that adds telomere DNA to chromosome ends, telomeres are reduced by 40–50 nucleotide pairs during each round of cell division.^{6–11} Telomeres are frequently shorter in tumors than in paired normal tissue,^{10–14} ostensibly reflecting the greater number of cell divisions that the tumor cells have undergone. Unprotected chromosome ends are highly recombinogenic, and once telomere shortening results in the formation of a chromosome with a critically short telomere, subsequent rounds of chromosome replication, chromatid fusion and unequal breakage can lead to the random loss, duplication or amplification of portions of the genome.^{4,9,15–18} Accordingly, we recently demonstrated that reduced telomere DNA content is associated with aneuploidy in human invasive ductal carcinoma of the breast.¹⁹

Excessively short telomeres have been associated with markers of poor prognosis in lung carcinoma, neuroblastoma, leukemia, endometrial and breast cancer.^{18–23} Previous studies also have shown that telomere length is reduced and that telomerase activity is elevated in prostate adenocarcinoma relative to normal prostate or benign hyperplasia of the prostate.^{24–26} The methodology used to measure telomere length in prior studies of cancer, an application of Southern blotting, requires microgram quantities of DNA and yields unreliable telomere length estimates when DNA is broken. Therefore archival tissue has not been used for retrospective studies of the relationship between telomere shortening and clinical outcome.

We have described a robust assay that quantitates telomere DNA content in as little as 16 ng of genomic DNA and

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* Requests for reprints: Department of Biochemistry and Molecular Biology, University of New Mexico School of Medicine, Albuquerque NM 87131-5221.

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is unaffected by DNA breakage,²⁷ permitting analysis of telomere DNA content in fresh, frozen and paraffin-embedded archival tissues. This assay was used in the present study to investigate the relationship between the content of telomere DNA in prostatic adenocarcinomas and clinical outcome as defined by survival and disease recurrence. The contents of tumor telomere DNA from patients who either died or had recurring disease within ten years of diagnosis and, in most instances, prostatectomy, were significantly less than in tumors from patients who survived and remained disease-free. The potential value of telomere DNA content as a prognostic marker in advanced prostatic adenocarcinoma is discussed.

MATERIALS AND METHODS

Study group. Power analysis indicated that a sample size of 18 would discriminate statistically significant differences in telomere DNA content in surviving and non-surviving men. Therefore, anonymous patient histories in the New Mexico Tumor Registry (NMTR), a population-based cancer registry, were searched to identify eighteen men diagnosed with prostatic adenocarcinoma prior to 1993. The group was selected so that approximately one half died within ten years of diagnosis and the other half survived at least ten years. The clinical characteristics of each member of the study population are shown in table 1 and summarized in table 2.

Source of prostate tissue. The NMTR links its database of anonymous patient histories to the locations of archival paraffin-embedded tumor and biopsy specimens through hospital pathology reference numbers, as approved by the University of New Mexico Health Science Center Human Research Review Committee. Archival, paraffin-embedded tissue was recovered for each patient. Tissue sections 25 microns thick were cut from the blocks. Histologically confirmed adenocarcinoma in prostatectomy specimens was separated from peripheral prostate tissue by gross dissection of tissue blocks guided by slides.

DNA purification. DNA was extracted from four sections using the QuiaAmp Tissue Kit (Quiagen Corp., Chatsworth,

CA) following the manufacturers directions. HeLa and placental DNA standards were prepared as described.²⁷

Measurement of telomere DNA content. Telomere DNA contents were determined as described.²⁷ Briefly, DNA samples were diluted, denatured and loaded on to duplicate nylon membranes. The membranes were washed, treated with 0.4 M NaOH to fix the DNA, sealed in plastic wrap, and stored at 4°C. Telomere and centromere specific oligonucleotides, end-labeled with γ -P³² ATP, were separately incubated with the duplicate membranes for 16 to 48 hr. under conditions producing 83–90% hybridization stringency. The membranes were then washed (83–90% hybridization stringency), air-dried and exposed to a storage phosphor screen (Molecular Dynamics) for 1–2 days. The quantities of the telomere and centromere probe that hybridized to the membranes were measured with a STORM PhosphorImager. These quantities were used to compute the ratio of telomeric DNA content to centromeric DNA content (T/C ratio) for each sample (normalizing the telomere DNA content to the centromere content compensates for abnormal DNA ploidy). The T/C ratio for each sample was expressed as a percentage of the T/C ratio determined from several dilutions of a reference DNA, purified from normal placenta, which was included on each blot. Several dilutions of a second reference DNA, which was purified from cultured HeLa cells and shown by Southern blot analysis to contain 53% of the telomere content of placental DNA,²⁷ was also included on each blot as an internal control. These reference DNAs provide means to establish the linear range of the assay and to normalize independent slot blot assays for batch-to-batch differences in the specific radioactivities of the centromere and telomere probes.

Telomere DNA content was used to divide the study group into two equal sub groups. Men with telomere DNA content less than the normal placental control comprise Group I. Men with telomere DNA content greater than placenta comprise Group II.

Statistical methods. The associations between telomere DNA content and both disease recurrence and ten year sur-

TABLE 1. Clinical data for study group

Patient Number	Age @ Dx ¹	PSA @ Dx	Year of Surgery	Gleason Score	Tumor Stage	Node Status	PSA @ PO ²	Vital Status ³	Telomere Content ⁴
869	72	56.1	1990	7	5	—	186	DOD: 3/97	45
556	66	2.8	NP ⁵	ND ⁶	ND	—	ND	DOD: 3/94	52
274	68	17	1991	9	6	—	51	DOD: 1/94	57
988	74	NA ⁷	NP	ND	ND	—	ND	DOD: 2/90	60
404	68	NA	1991	7	6	—	125	DOD: 6/95	65
716	67	16.5	1990	7	4	—	8.4	LDI: 3/99	67
859	61	49	1990	7	6	—	10.7	LDI: 4/99	70
887	61	11	1990	7	6	—	7	DOD: 5/94	70
616	68	NA	1986	8	3	—	5.3	DOD: 12/94	92
867	70	NA	1986	7	6	—	2.4	DOD: 4/97	123
254	64	18	1992	7	4	—	0.8	LDI: 4/98	128
809	69	NA	1990	7	6	—	0.1	LDI: 4/96	144
053	76	15	1991	7	5	—	0.5	LDI: 2/98	149
853	68	29.5	1991	8	6	—	2.2	LDI: 3/99	150
494	72	6.9	1993	9	6	—	0.1	LDI: 4/98	245
742	74	9.5	1993	9	6	+	0.3	LDI: 2/99	277
599	66	4.2	1991	5	ND	—	0.3	LDI: 1/98	291
905	70	6.5	1992	7	ND	—	0.9	LDI: 4/98	485

1) Dx: diagnosis, 2) Last PSA after prostatectomy, 3) DOD: date of death; LDI: last date known alive, 4) Expressed as percentage of placental DNA standard, 5) NP: not performed, 6) ND: not determined, 7) NA: not available.

TABLE 2. Relationship between telomere DNA content and outcome in prostate cancer

Group	N	Telomere Content			Age @ Dx			Survival @ 10 Yrs	Tumor Stage			Gleason Score			PSA @ FU		
		M	R	CI	M	R	CI		M	R	CI	M	R	CI	M	R	CI
All	18	108	45–485%	0–339%	68	61–74	68–69	11/18	6	3–6	5–6	7	5–9	7.3–7.4	2.3	0.1–186	0–109
I	9	65	45–92%	60–68%	68	61–74	66–67	2/9	6	3–6	5–6	7	7–9	7.3–7.4	10.7	5.3–186	0–133
II	9	150	123–485%	0–518%	70	64–74	69–70	9/9	6	4–6	5–6	7	5–9	7.2–7.3	0.5	0.1–2.4	0.8–0.9

1) Median values, range and the 95% confidence interval are reported for each parameter. 2) For pathological stage, 3 = T2a, 4 = T2b, 5 = T2c and 6 = T3a. 3) Telomere DNA content is expressed as a percent of placental DNA standards included on each blot. Abbreviations: M, median, R, range, CI, 95% confidence interval, Dx, diagnosis, FU, follow up.

This looks rather squished to me

vival were determined by two sample, two-sided T-test with unequal variance with Microsoft Excel 97 software. Kaplan-Meier plots were generated by JMPIN, version 3 (SAS Institute, Inc).

RESULTS

The characteristics of the study group are summarized in table 1. Typically, tumors were node-negative, organ confined (stage T2c or T3a), and moderately well differentiated (average Gleason sum score: 7.4). The contents of telomere DNA purified from the 18 specimens varied from 45–48% of that of the placental DNA standard included on each blot (table 1). The mean telomere DNA contents in 21 samples of peripheral "normal" prostate tissue, and 10 samples of normal tissue derived from other anatomical sites, including kidney, peripheral blood lymphocytes, lymph nodes, bone marrow and breast were 92% and 95%, respectively, of the placental standard (data not shown). The placental reference DNA serves as a basis for comparison to normal tissues.

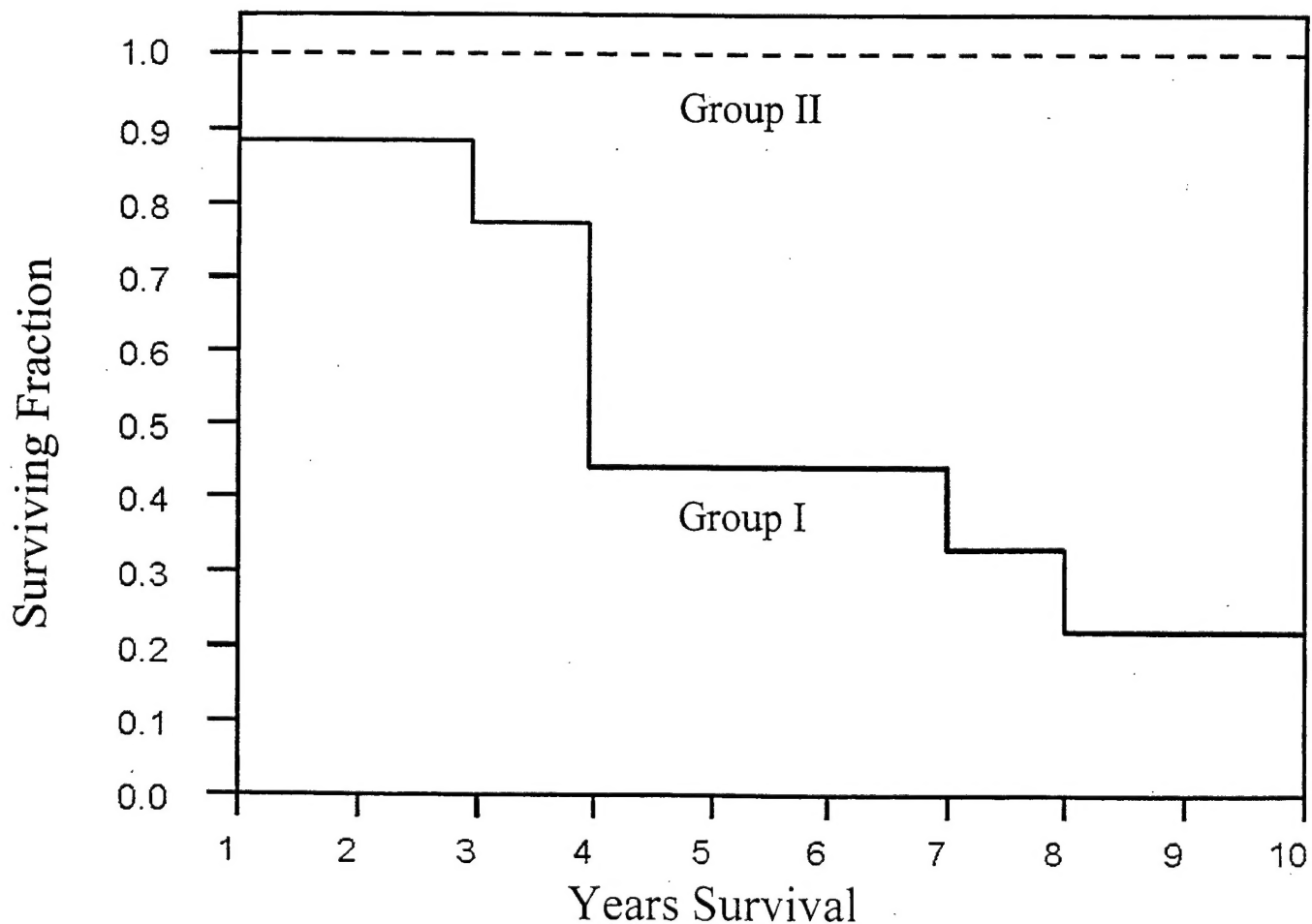
Tumors were divided into two equal groups based on telomere DNA contents. The samples in Group I had telomere DNA contents that were less than in the placental DNA, while the telomere DNA contents in Group II exceeded the placental control. There was no other apparent differences in the two groups, including mean age, tumor stage and Gleason sum score (table 2). The relationship between telomere DNA content and survival within the two groups is shown in figure 1. Seven of the nine patients in Group I, but none of nine men in Group II died within ten years of diagnosis. The Fisher Exact Test indicated that the difference in the pa-

tients' survival in the two groups was highly significant ($p < 0.0001$).

The relationship between telomere DNA content and disease recurrence (arbitrarily defined as PSA levels of >2.5) within the subsets of men in Groups I and II that had prostatectomies is shown in figure 2. (Two of the patients in Group I did not have prostatectomies because their medical status precluded surgery.) All seven patients who had prostatectomies had recurrent disease within ten years of surgery. In contrast, all of the patients in Group II, who all had prostatectomies, remained disease free for at least ten years after surgery. The Fisher Exact Test indicated that the difference in disease recurrence in the two groups was also highly significant ($p < 0.0001$). There was no apparent relationship between telomere DNA content and other prognostic factors for prostatic adenocarcinoma, including patient's age at diagnosis, node status, pathological grade or Gleason sum score (table 2).

DISCUSSION

The principal finding of this preliminary study is that there was a highly significant association in patients with advanced prostate cancer between telomere DNA content and each of two measures of clinical outcome, mortality and disease recurrence. Seven of the nine men in Group I, but none of the nine men in Group II, died within ten years of diagnosis of prostate adenocarcinoma. From the records available, we cannot determine whether prostate cancer caused or contributed to the death of all of these men. However, of the sixteen men in the study group who had pros-



F2

PSA at Follow Up

REFERENCES

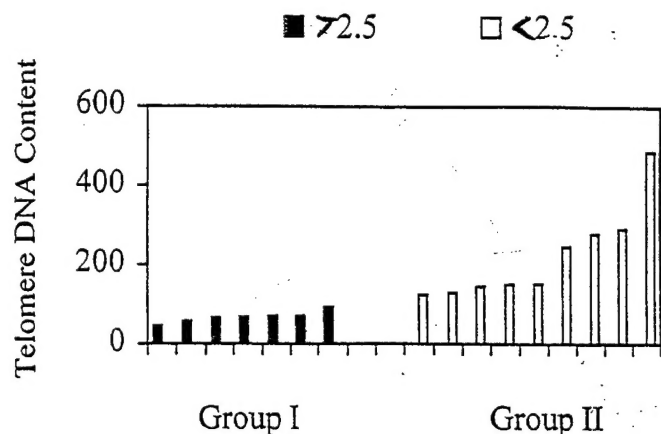


FIG. 2.

tatectomies, all seven in Group I had PSA levels in excess of 5.0 at follow up (mean 56, median 31), indicating the recurrence of their disease. In contrast none of the nine men in Group II had a PSA level above 2.5 (mean 0.8, median 0.5). Therefore, we think it likely that the difference in survival of the patients was at least in part due to recurrence of their prostate cancers. In this context, the significant association between outcome and telomere DNA content is remarkable.

Telomerase is the ribonucleoprotein that adds telomeric repeats to the ends of chromosomes. Normally telomerase is expressed in only a few highly proliferative tissues. However, extensive investigations have demonstrated that telomerase is active in nearly all cancers, including prostate adenocarcinoma.^{24-26,28,29} Interestingly, a recent study by Koenen and colleagues shows that telomerase expression is detected in only a fraction of prostatic intraepithelial neoplasia (PIN).³⁰ The difference in telomere length seen in the two Groups of this study may reflect an earlier up regulation of telomerase expression in Group II, where telomere DNA content is greater.

Although telomere shortening has been associated with markers of poor prognosis in previous studies of cancer,¹⁹⁻²³ to the authors' knowledge, this is the first study in which the relationship between telomere shortening and clinical outcome has been investigated. It is well documented that excessive telomere shortening leads to genomic instability, and gain or loss of genomic DNA is ubiquitous in metastatic prostate cancer. Taken together, these data imply that the rate or extent of telomere shortening is an important determinant of clinical outcome.

The present study was performed with archival tissues derived from radical prostatectomies and core biopsies, demonstrating the feasibility of exploiting archival tissue to perform retrospective studies of telomere DNA content. In a recent study,³¹ sampling six random systematic core biopsies (SRCSB) detected clinical prostate cancer in 80% of patients. Given SRCSBs ability to detect prostate cancer, and the potential of telomere DNA content to predict disease mortality and recurrence suggested by the current study, we believe that the prognostic value of telomere DNA content warrants further investigation.

CONCLUSIONS

Telomere DNA content may differentiate high-risk patients with metastatic prostate cancer from men with indolent disease who can be spared the unnecessary side effects and expense of treatment by management with "watchful waiting."

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